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Detection of Satratoxin G and H in Indoor Air from a Water-Damaged Building

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Abstract The occurrence of *Stachybotrys chartarum* in indoor environments has been linked to adverse health effects as well as few cases of pulmonary haemorrhages in humans. Although the highly toxic secondary metabolites of this fungus, like satratoxin G and H, were frequently claimed with outbreaks of such diseases, these toxins have hardly been identified in the air of naturally contaminated indoor environments. Herein, a case of a LC–MS/MS-confirmed occurrence of airborne *S. chartarum*-toxins in a water-damaged dwelling is reported. Satratoxin G (0.25 ng/m^3) and satratoxin H (0.43 ng/m^3) were detected. This provides further evidence that *Stachybotrys*-toxins can be transferred from mouldy indoor materials into air, which could be a factor in the aetiology of health symptoms related to the sick building syndrome.

Keywords Indoor air · LC–MS/MS · Macrocyclic trichothecenes · Satratoxin · Sick building syndrome · *Stachybotrys chartarum*

Abbreviations

MVOCs	Microbial volatile organic compounds
SBS	Sick building syndrome
SG	Satratoxin G
SH	Satratoxin H
VOCs	Volatile organic compounds

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Introduction

Macrocyclic trichothecenes produced by *Stachybotrys chartarum* are highly toxic compounds associated with the so-called 'sick building syndrome' (SBS) in humans. Several case control studies have shown that the occurrence of *Stachybotrys chartarum* in indoor environments could be related to headaches, fatigue, nausea, vomiting, haemorrhage, depression, sleep disturbances, anxiety, vertigo, memory-loss and even cases of idiopathic pulmonary haemosiderosis suffered by children from these environments [1–5]. *S. chartarum*, a well-known producer of macrocyclic trichothecenes [6], can grow and produce mycotoxins on humid cellulose-containing materials like wallpaper or gypsum board (e.g. after water damages) [1, 7–10], while optimal growth conditions for this mold are given at a water activity value of 0.98 [11]. Elidemir et al. [12] reported the first case of an isolation of *Stachybotrys chartarum* in the lung of a child suffering from pulmonary haemosiderosis. Recently, albumin-adducts of satratoxin G were discussed to serve as biomarkers for an exposure to *Stachybotrys chartarum*. They were observed in sera of rats and cats after exposition to this fungus [13, 14]. However, a causal relationship between *S. chartarum* and illnesses observed in sick buildings is not yet proven, since there are a variety of other pollutants like the wide range of VOCs, MVOCs, endotoxins, respirable dusts and other compounds in the indoor environment, which also can be a reason for adverse health effects [15, 16]. Findings of Johanning

et al. [17] and Bloom et al. [18] gave evidence that these toxins can become airborne. To our knowledge, the case described here is the first report of a LC–MS/MS-confirmed occurrence of airborne satratoxins in a naturally contaminated dwelling.

Materials and Methods

Sampling

Sampling was conducted in a dwelling with water damage and well-known occurrence of *Stachybotrys* and its toxins [10]. Satratoxin G (SG) and satratoxin H (SH) had previously been found in the mouldy wallpaper of this dwelling with 9.7 and 12 μg/cm^2, respectively. For indoor air sampling, a MD 8 air sampler (Sartorius, Goettingen, Germany) was used. Air was filtered through a polycarbonate membrane filter with a pore size of 0.8 μm (Millipore, Schwalbach, Germany) during 15 h at a flow rate of 5 m^3/h.

Chemicals and Reagents

Standards of satratoxin G and H were kindly provided by Prof. Gareis (Max Rubner-Institut, Kulmbach, Germany). Acetonitrile and methanol (HPLC-grade) were purchased from J.T. Baker (Griesheim, Germany). LC–MS quality ammonium formate (Fluka, Deisenhofen, Germany) and water purified in a milli-Q water purification system (Millipore, Schwalbach, Germany) were used.

Sample Preparation and Recovery Experiments

The polycarbonate filter membrane (80 mm diameter) was cut into small pieces and extracted with 10 ml acetonitrile/deionised water 84/16 (v/v) on a horizontal shaker for 30 min. The extract was centrifuged for 10 min at 6,000*g* and cleaned by passing 5 ml through a Bond Elut Mycotoxin® column (Varian, Darmstadt, Germany). Three millilitres of filtrate was evaporated under a gentle stream of nitrogen at 40°C (Barkey, Leopoldshöhe, Germany). Residues were dissolved in 500 μl methanol/deionised water 1/9 (v/v) and filtered through a 0.45 μm PTFE syringe filter (Supelco, Deisenhofen, Germany).

For testing the recovery of SG and SH from polycarbonate filters, blank membranes (80 mm diameter; $n = 3$) were contaminated with 50 ng of each toxin and extracted according to the method above. Furthermore, a blank filter membrane and an air sample from a *Stachybotrys*-free indoor environment were used as negative controls.

LC–MS/MS Analysis

The LC–MS/MS measurements were done with a HPLC apparatus Series 200 (Perkin-Elmer, Rodgau-Jügesheim, Germany) and an API 3200 triple quadrupole mass spectrometer (Applied Biosystems, Darmstadt, Germany). A Gemini 150 × 2 mm, 5 μm (Phenomenex, Aschaffenburg, Germany) was used as the analytical column. The binary linear gradient consisted of eluent A (deionised water + 5 mM ammonium formate) and eluent B (methanol + 5 mM ammonium formate) with a flow rate of 400 μl/min: 0 min 10% B, 8 min 100% B, 12 min 100% B, 12.5 min 10% B, 15 min 10% B. The column oven temperature was maintained at 40°C and the injection volume was 20 μl.

The MS/MS experiments were carried out in positive electrospray ionisation mode (ESI+) with an ion spray voltage (ISV) of 4,000 V and a source temperature of 300°C. The nebulizer gas flow was set at 50 psi, the heating gas at 30 psi and the curtain gas at 20 psi. The CAD (collisionally activated dissociation) nitrogen gas flow was used in high mode.

The toxins were identified in multiple reaction-monitoring mode (MRM). SG and SH showed the best sensitivity as adduct-ions of ammonium $[M+NH_4]^+$. The fragment ions were selected from mass spectra of product ion scans of SG and SH which are shown in Figs. 1 and 2, respectively. The fragment ion *m/z* 249.1 indicated the cleavage of the macrocyclic ring between C4 and C15 of the molecule. It was the most intensive fragment for SG, but also occurred after fragmentation of SH. Nielsen et al. [19] also described this product ion after fragmentation of another trichothecene, trichoverrol A. Another fragment ion of SG and SH, *m/z* 231.1, which is also a common product of all trichothecenes [20], resulted from a further loss of H_2O. Other major fragments of SG, like *m/z* 133.2 or 105.2, could not be interpreted. For SH, the product ion *m/z* 245.2 was the most sensitive and selective fragment, but with unknown structure.

For the identification of SG, the following optimised parameters and two mass transitions (one

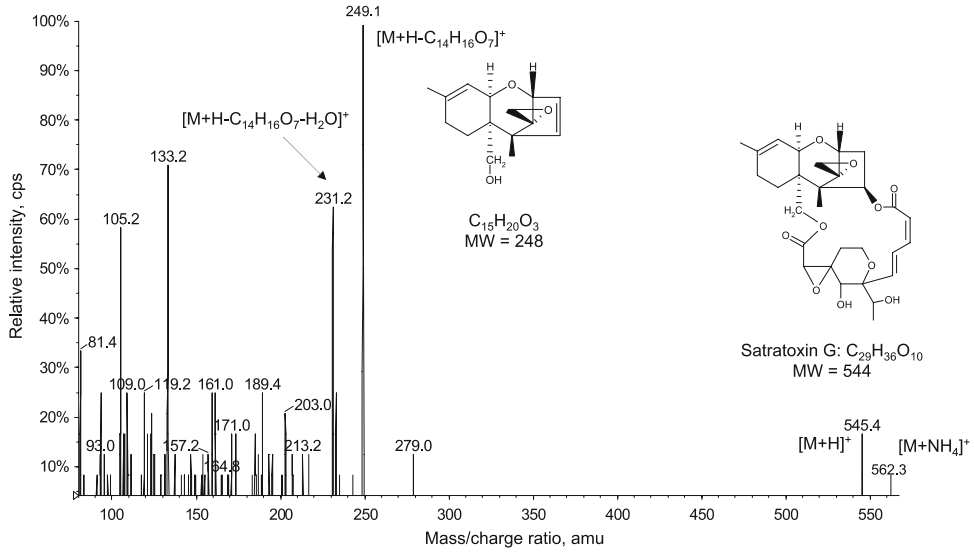

Fig. 1 Product ion spectrum (LC–MS/MS) of SG based on the precursor ion $[M+NH_4]^+$. Ionisation ESI+, ISV 4,000 V, DP 21 V

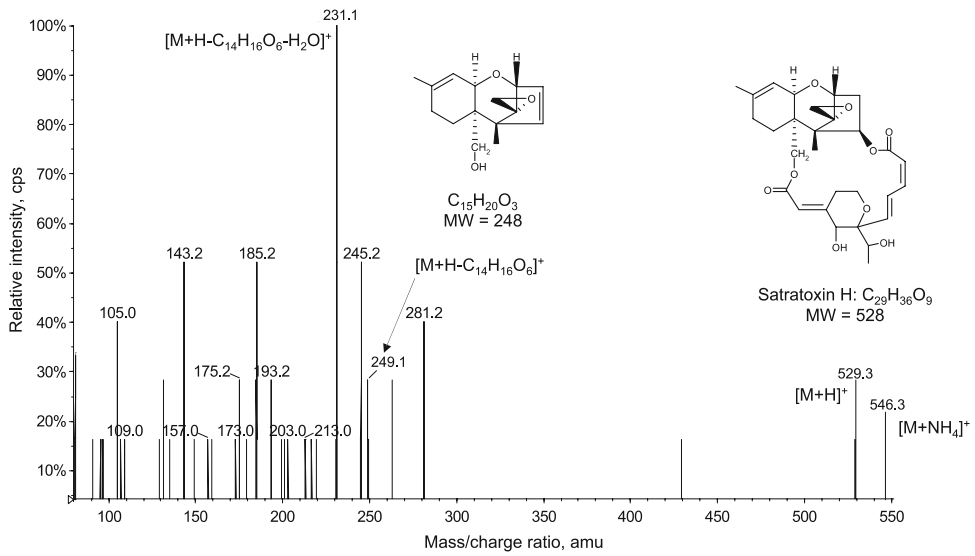

Fig. 2 Product ion spectrum (LC–MS/MS) of SH based on the precursor ion $[M+NH_4]^+$. Ionisation ESI+, ISV 4,000 V, DP 21 V

quantifier and one qualifier) were used: declustering potential (DP) 21 V, *m/z* 562.3/249.1 (collision energy (CE): 19 eV), and *m/z* 562.3/231.1 (CE: 21 eV). For SH: DP: 21 V, *m/z* 546.3/245.2 (CE: 25 eV), and *m/z* 546.3/231.1 (CE: 25 eV).

Results and Discussion

The recovery of SG and SH from polycarbonate filter membranes has been tested on one level (50 ng) with three replicates. Recovery rates (mean ± RSD) of 68 ± 3.4 and 56 ± 4.8% were obtained for SG and SH, respectively. No signals were observed after measurement of the control samples. Hence, this method was considered as suitable for indoor toxin measurements.

The indoor air of the *Stachybotrys*-affected dwelling was collected during 15 h at a flow rate of 5 m^3/h, corresponding to 75 m^3 total filtered air. A total toxin content of 19 ng SG and 32 ng SH was determined on the polycarbonate filter membrane by LC–MS/MS

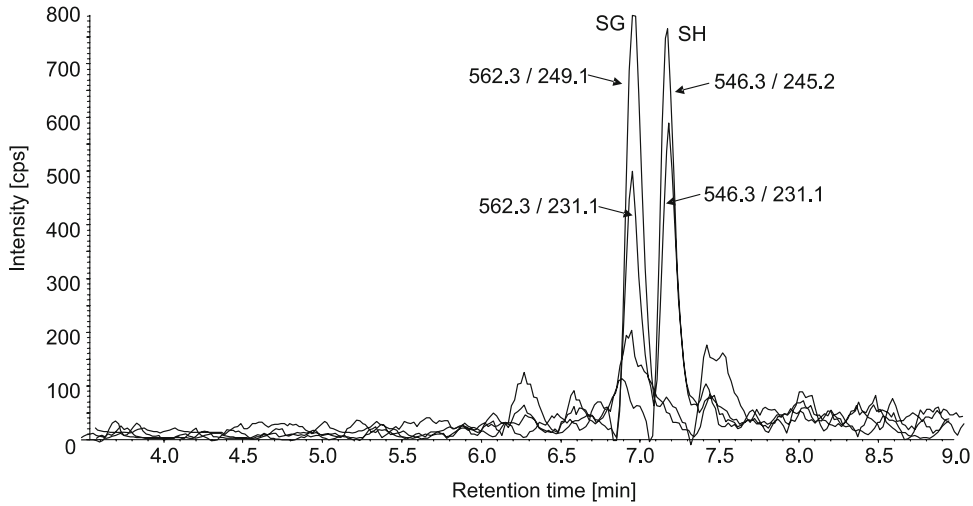

Fig. 3 MRM-chromatogram (LC–MS/MS) of SG and SH extracted from air filter membrane

(see chromatogram in Fig. 3), corresponding to a concentration of 0.25 ng/m^3 SG and 0.43 ng/m^3 SH in the indoor air. With regard to these concentrations, a total amount of 2.0 ng SG + SH would have been inhaled during a stay of 8 h in this environment (respiratory minute volume: 6 l, at rest).

Sorenson et al. [21] first showed the possible inhalation of satratoxins after an experiment with artificially aerosolized conidia of *S. chartarum* in a laboratory model. Dust collected on glass-fibre filters consisted of more than 85% of conidia of *S. chartarum* and contained SG, SH and trichoverrols. Later, Brasel et al. [22] showed that macrocyclic trichothecenes could exist on fungal fragments even smaller than conidia. Recently, they also examined airborne macrocyclic trichothecenes in *S. chartarum*-contaminated buildings [23]. The results of ELISA showed toxin levels between 0.01 and 1.3 ng/m^3 in sampled air. These levels are comparable to the LC–MS/MS confirmed concentrations of SG and SH in the present case. Furthermore, because of the concurrent detection in the wallpaper [10], it could be proven that *Stachybotrys* toxins actually were transferred from mouldy indoor materials into indoor air. Whether these concentrations are high enough to contribute to the diseases of the sick-building syndromes' complex must be concluded after further study.

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